Among-Sampler Variation in Sweep Net Samples of Adult *Lygus hesperus* (Hemiptera: Miridae) in Cotton

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ABSTRACT   The sweep net is a standard sampling method for adults of the western tarnished plant bug, *Lygus hesperus* Knight (Hemiptera: Miridae), in cotton (*Gossypium* spp.). However, factors that influence the relationship between true population levels and population estimates obtained using the sweep net are poorly documented. Improved understanding of these factors is needed for the development and application of refined treatment thresholds. Recent reports of significant among-sampler differences in sweep net-based population estimates of the adult tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), seem to preclude meaningful comparisons of population estimates collected by different samplers. We used a mark–release–recapture method and the standard sweep net to evaluate among-sampler differences in population estimates of *L. hesperus* adults. Adult lygus, marked with fingernail polish to facilitate identification and prevent flight, were released into 10-m sample rows on the evening before 10-sweep samples were collected the following morning. The experimental design was a randomized complete block with three replications of three treatments (sampler). Separate experiments were conducted in two plantings each of Pima (*Gossypium barbadense* L.) and Acala (*Gossypium hirsutum* L.) cotton. Collections of marked bugs from each study were evaluated for effects of sampler, sample date, and their interaction. Although differences in lygus collections were observed among sample dates in some tests, no differences were detected in the population estimates by different samplers. These results demonstrate that the sweep net technique can be sufficiently standardized to allow direct comparison of population estimates obtained by different samplers.

KEY WORDS   sampling, cotton, sweep net, plant bug

The western tarnished plant bug, *Lygus hesperus* Knight (Hemiptera: Miridae), and other lygus species are key pests of cotton (*Gossypium* spp.) in the arid western United States. The lygus complex has also become increasingly important in other U.S. cotton production regions. The elevated pest status of lygus bugs in much of the Cotton Belt has prompted research to improve management strategies. However, efforts to develop effective lygus management rules are hampered by the uncertain interpretation of population estimates provided by commonly used relative sampling methods. Improved knowledge of the relationships between estimates of lygus populations and their true densities would facilitate the design and interpretation of studies of cotton responses to lygus infestation.

Numerous studies of sweep net sampling for lygus have incorporated one or more absolute sampling methods for comparison (Byerly et al. 1978, Ellington et al. 1984, Fleischer et al. 1985, Snodgrass and Scott 1997, Zink and Rosenheim 2004). However, results of those studies are difficult to interpret because of the variation among studies in the methods of sweeping. More recent studies focusing on relative sampling methods have made no attempt to relate population estimates by those methods to true population densities (Gore and Catchot 2005, Sharp and Bagwell 2006, Stewart et al. 2006, Musser et al. 2007). A notable finding by Musser et al. (2007) was that different samplers using the sweep net in cotton obtained statistically different population estimates of the tarnished plant bug. If that conclusion is valid, it seems unlikely that reliable and widely applicable treatment thresholds could be developed on the basis of samples collected by the sweep net. Furthermore, interpretation of results of research conducted by different investigators or involving multiple samplers would not be straightforward. One potential shortcoming of the study by Musser et al. (2007) is that different samplers obtained estimates of lygus populations in different fields or at different sites within fields. Therefore, it seems likely that at least some of the variability at-

tributed to differences among samplers was a reflection of the different lygus population levels sampled.

Spurgeon (2009) developed a mark–release–recapture approach for evaluating the sweep net as a sampling method for adult lygus in cotton. This approach permits the investigator to collect samples from known lygus population levels, thereby alleviating many of the problems associated with the collection of absolute population estimates, and reducing population heterogeneity among sampled areas. We used these mark–release–recapture methods to evaluate among-sampler differences in collections of adult *L. hesperus* from both Upland (Acala) (*Gossypium hirsutum* L.) and Pima (*Gossypium barbadense* L.) cotton.

Materials and Methods

We conducted a total of four experiments in two separate plantings of each the Acala variety 'Phytogen 72′ and the Pima variety 'Phytogen 800′ (Dow Agro-Sciences, Indianapolis, IN) on the Shafter Research and Extension Center, Shafter, CA. In the first planting, the two cotton types were planted in adjacent areas of the same field in late April. The two cotton types were also planted adjacent to each other in the second planting (early June) but in a different field from the first planting. All plantings used a 1.02-m row spacing. For simplicity we refer to these experiments according to planting (experiment 1, Acala or Pima; experiment 2, Acala or Pima). Within each combination of planting and cotton type, 3–5 sampling areas (blocks) were established, from which three blocks were selected for use on each sample date. Each block was composed of 12–16 parallel 10-m row sections. The ends of each of these row sections were separated from the remainder of the row by a buffer area ($\geq$1 m) from which the plants were removed.

Marking Adult Lygus. Lygus adults for release were obtained from a laboratory colony reared on pods of green bean, *Phaseolus vulgaris* L., and raw sunflower, *Helianthus annuus* L., seeds or were collected from plots of alfalfa, *Medicago sativa* L. Lygus adults were harvested from the colony three times weekly. Rosenheim et al. (2004) reported apparent gender-based differences in the plant part associations of lygus adults. Cooper and Spurgeon (2010b) reported differences in feeding behaviors among classes of adult lygus characterized by age, reproductive development, and mating status. Because little is known regarding the age distribution of naturally occurring lygus in cotton or whether such age distribution is stable, when adequate numbers of adults were available from each cohort, four age classes (2–5, 5–7, 7–9, and 9–12 d old) were marked and released into each sampled row in equal numbers. When numbers of adults of any age cohort were insufficient to accommodate releases into all rows sampled on a given date, field-collected bugs of unknown ages were substituted for those age classes. Overall, $\approx$45% of released bugs originated from the laboratory colony.

Lygus adults, whether they were obtained from the colony or field, were held for $\geq$24 h before they were marked. To facilitate marking, small aliquots of five to 10 bugs were aspirated into plastic vials and lightly anesthetized with CO_2. Anesthetized bugs were decanted into a petri plate bottom which was lined with moist filter paper. Each bug was oriented so a small droplet of fingernail polish could be applied to the dorsum near the posterior of the scutellum. After bugs were marked the petri plate was closed by a screened lid and set aside until the polish was dried. Marked bugs that were able to separate their wings or that had legs, antennae, or heads coated with polish were discarded. Remaining marked bugs were held in same-age groups of 200–400 individuals within 3.8-liter plastic buckets closed with a screened lid and containing shredded paper and fresh green bean pods. Marked bugs originating from the colony were held in environmental chambers at 24°C and a photoperiod of 14:10 (L:D) h until they were released. Marked bugs originating from the field were held in the laboratory at room temperature. A different color of mark was used on each sample date within each planting.

Lygus Releases and Sampling. Each week, three 10-m rows from each of three sampling areas (blocks) in each cotton type were selected for sampling marked and released lygus adults. Sampling rows within each block were selected on the basis of similarity in plant size and the absence of large ($>$1-m) skips in the plant stand. Each of three samplers was then randomly assigned to one row in each of the three blocks. In addition, two 1-m sections of row separated from adjacent plants by $\geq$1 m were established adjacent to the sample areas in each cotton type. These short row sections, which were selected based on similarity to the sampled rows, were used to estimate the degree to which released bugs remained in the sampled rows (Spurgeon 2009).

On the evening before sampling, marked bugs were aspirated into 44.4-ml (12-dram) plastic vials (no. 55-112, Thornton Plastics, Salt Lake City, UT) for transport to the field. Each of the 80 labeled vials contained 10 bugs of a given age class and was closed with a snap-cap lid. Each vial lid was penetrated by a hole ($\approx$0.8 cm in diameter) that was closed with a rubber stopper. The vials were then sorted so that 10 bugs of each age class (40 bugs in total) were assigned to each sample row. Beginning after 1900 hours (PDT), 40 marked bugs were released into each of the three selected 10-m sample rows per block. This population density was selected based on the results of Spurgeon (2009) to minimize the occurrence of zero counts, which would have diminished the statistical power of planned comparisons. Marked bugs were released onto plant terminals and upper leaves and were spaced as evenly as possible down each row. Immediately after releases into sample rows, 12 bugs (three bugs of each age class) were released into each 1-m row section used to monitor retention of marked bugs. In both cotton types combined on a given sample date, 720 marked bugs in total were released into the sample rows, and an additional 48 bugs were released into the

1-m row sections. The remainder of the 800 marked bugs was used to replace any bugs that were dead by the time of release.

The three samplers varied in experience with the sweep net. One sampler had extensive experience sweeping lygus in cotton and alfalfa by using a one-handed technique. One sampler was experienced with the sweep net in cotton by using a two-handed technique. The third sampler was experienced with the sweep net, but for sampling insects other than lygus and in habitats other than cotton. Immediately before the first samples were collected, a 15-min orientation was conducted to ensure the three samplers used the same technique with a standard 38-cm-diameter sweep net. The samplers took pendulum sweeps by using a two-handed technique similar to that described by Godfrey et al. (2010). One pass of the net through the upper 20–25 cm (8–10 inches) of the plant canopy constituted a single sweep. During the orientation a stopwatch was used to ensure that each sampler could take 10 sweeps in 6–7 s.

Between 0900 and 1000 hours on the morning following bug releases, each 10-m row was sampled by 10 sweeps. Collected bugs from each 10-sweep sample were placed in a sealable plastic bag, which was transferred to the laboratory where both marked (released) and unmarked (naturally occurring) lygus adults were counted. Plants in the 1-m row sections were cut into pieces and visually searched for marked bugs, which were recorded as alive or dead.

Immediately after sampling, five plants from locations evenly spaced down each sampled row were examined to characterize crop development. Measurements recorded included plant height (mainstem length from the soil surface to the terminal), mainstem node number (considering the hypocotyl as node 0 and counting to the uppermost expanded leaf), canopy width, and phenological stage (vegetative, sub-pinhead, pinhead, matchhead, and 1/3-grown square, candle, white bloom, and boll). Squaring stages were distinguished by the diameter of the developing bud within the bracteoles. The bud of a sub-pinhead square was <1 mm in diameter. Buds ≥1 mm in diameter but <3 mm were classed as pinhead. Buds of matchhead squares were ≥3 mm in diameter but <6 mm in diameter but without the elongated petals characteristic of candles were classed as 1/3-grown.

Statistical Analyses. All analyses were conducted using SAS (SAS version 9.2, SAS Institute, Cary, NC). Plant development for each cotton type on each sample date was characterized by the means of plant height, canopy width, and node number, and the median of phenological stage. Collections of both marked (released) and unmarked (naturally occurring and of unknown population density) lygus adults were analyzed separately for each experiment (combination of planting and cotton type) by using mixed model analysis of variance (ANOVA, PROC GLIMMIX). Each analysis included fixed effects of sampler, date, and their interaction, and the random effect of block. In addition, the numbers of marked bugs recovered alive from the 1-m row sections were compared among dates within experiments and cotton types by one-way ANOVA using PROC GLIMMIX. In each analysis, where differences among levels of a fixed effect were indicated, means were compared controlling the experiment-wise type I error rate with the ADJUST = SIMULATE option of the LSMEANS statement.

Spurgeon (2009) indicated that collection efficiency of the sweep net for lygus adults decreased when plant heights exceeded ≈50 cm. Although changes in sweep net collection efficiency were associated with plant height, the specific plant characteristic(s) responsible for these changes were not identified. If a reliable relationship between collection efficiency and one or more plant parameters could be established, those plant parameters would have considerable utility as covariates in future studies of sampling methods and population dynamics. Therefore, the influences of plant characteristics on sweep net captures of marked lygus were examined through preliminary mixed model ANOVAs. In these analyses the mean plant height, canopy width, or ratio of plant height to width corresponding to each sampled row was substituted as a proxy for the fixed effect of date in the previously described ANOVA models. Because sample date represents a composite of individual factors influencing collection efficiency, including the plant parameters and other factors such as temperature, humidity, wind speed, and light intensity, we considered it the most effective manner of partitioning these sources of variance. Evaluations of models containing plant parameters as proxies for date were subjective and were based on the similarity of model output to the output of models containing date as an effect.

Results

Experiment 1, Acala Cotton. Sampling was not initiated until plants averaged 10 nodes and >40 cm in height because early square retention was poor. Over the duration of the experiment, average plant heights corresponding to different sample dates ranged from 43.5 cm on 10 June to 83.7 cm on 1 July (Table 1). Canopy width was generally less than plant height (height:width ranged from 1.11 to 1.38). In addition, a median phenological stage of 'boll' was not attained until 8 July (Table 1). The relatively narrow canopy width and extended preflower squaring period were probably indicative of poor fruit retention until the last weeks of the experiment. One consequence of poor fruit retention was a concentration of squares in the upper portions of the plants.

ANOVA did not indicate differences among samplers in the numbers of marked lygus that were recovered by the sweep net ($F = 0.44$; df = 2, 32.3; $P = 0.65$). The absence of a significant sampler by date interaction ($F = 1.27$; df = 10, 32.3; $P = 0.29$) indicated the lack of differences among samplers was consistent across sample dates. However, the numbers of marked lygus that were recovered differed among sample dates ($F = 3.86$; df = 5, 24.72; $P = 0.01$). The mean of

Table 1. Plant parameters and numbers of marked and unmarked adult *L. hesperus* collected per 10-sweep samples from a late-April planting of Acala cotton

Date	Plant ht (mean ± SE)	No. nodes (mean ± SE)	Canopy width (mean ± SE)	Growth stage (median)	Marked bugs (mean ± SE)	Unmarked bugs (mean ± SE)
10 June	43.5 ± 0.65	10.2 ± 0.14	35.3 ± 0.74	Matchhead square	5.7 ± 0.52a	2.6 ± 0.59
17 June	52.4 ± 0.76	11.7 ± 0.17	46.1 ± 0.90	Matchhead square	3.4 ± 0.51b	2.2 ± 0.59
25 June	72.0 ± 0.77	14.0 ± 0.17	59.8 ± 1.71	1/3-grown square	3.5 ± 0.51ab	2.1 ± 0.59
1 July	83.7 ± 1.33	15.9 ± 0.21	67.7 ± 2.29	1/3-grown square	3.2 ± 0.51b	2.2 ± 0.59
8 July	58.3 ± 1.37	15.4 ± 0.22	43.6 ± 1.44	Boll	3.0 ± 0.50b	1.7 ± 0.59
22 July	80.4 ± 1.88	18.9 ± 0.35	74.2 ± 2.14	Boll	2.9 ± 0.50b	3.1 ± 0.59

Means in a column followed by the same letter are not significantly different ($P > 0.05$; SIMULATE option of the LSMEANS statement in SAS).

counts of marked bugs was higher on 10 June than on any other sample date except 25 June (Table 1). Retention of marked bugs in the sample rows, as indicated by the 1-m row sections, did not differ among sample dates ($F = 1.51$; df = 5, 6; $P = 0.31$). Numbers of marked bugs recovered alive ranged from 9.5 (79%, 25 June and 8 July) to 12 (100%, 17 June). Therefore, the higher numbers of marked bugs collected from the sample rows on 10 June was likely a consequence of the relatively small plant size, compared with other dates. Given the number of marked bugs released into each sample row and the diameter of the sweep net, if all released bugs remained on the row and sampling efficiency was 100% the mean expected capture was 15.2 bugs (Spurgeon 2009). Based on this expected value, collection efficiency of the sweep net (mean ± SE) ranged from $37.3 ± 2.69$ to $19.0 ± 2.78%$. Excluding the first sample date (10 June) the maximum estimated collection efficiency was $23.4 ± 3.31%$. As for the marked bugs, numbers of unmarked, naturally occurring lygus recovered by the sweep net did not vary significantly among samplers ($F = 0.97$; df = 2, 36; $P = 0.39$), and no sampler by date interaction was observed ($F = 0.42$; df = 10, 36; $P = 0.93$; Table 1). However, the numbers of native unmarked lygus also did not vary among sample dates ($F = 0.68$; df = 5, 36; $P = 0.64$; Table 1), which was in contrast to the results for marked and released bugs.

Experiment 1, Pima Cotton. As for the first planting of Acala cotton, sampling was not initiated in the first planting of Pima cotton until plants averaged nearly 10 mainstem nodes in development (Table 2). Although canopy width in the Pima planting was generally similar to plant height (height:width ranged from 0.91 to 1.25), the Pima planting also exhibited poor early fruit retention and a concentration of squares in the upper canopy. Because the Pima variety we used matures more slowly than the Acala variety, and both plantings exhibited similarly poor early fruit retention, plants in the Pima planting were generally less developed than those in the Acala planting. In fact, most plants in the Pima planting did not have one-thirds-grown squares or bolls until 1 July and 22 July, respectively.

Numbers of marked adult lygus collected by the sweep net were similar among samplers ($F = 0.48$; df = 2, 36; $P = 0.48$) but varied significantly among sample dates ($F = 4.14$; df = 5, 36; $P < 0.01$). The nonsignificant sampler by date interaction ($F = 1.02$; df = 10, 36; $P = 0.44$) indicated differences observed among dates were consistent among samplers. Numbers of marked lygus collected by the sweep net were higher on 10 and 17 June than on 8 July (Table 2). No differences among other dates were demonstrated. The general tendency was for numbers of recovered bugs to decrease with increasing plant size until ≈1 July. Numbers of marked bugs recovered alive from 1-m row sections did not differ among sample dates ($F = 0.33$; df = 5, 6; $P = 0.86$), and ranged from 10.0 (83%, 25 June, 8 and 22 July) to 11.5 (96%, 10 June and 1 July). Therefore, differences among dates in collections of marked bugs from sample rows were not likely to be caused by the loss of marked bugs on some dates. The steady decline in numbers of captured bugs was reflected in estimated collection efficiencies ranging from $39.5 ± 6.11%$ (10 June) to $15.4 ± 4.25%$ (8 July).

Sample counts of naturally occurring lygus also did not differ among samplers ($F = 1.35$; df = 2, 36; $P = 0.27$). Although collections of unmarked native bugs varied among sample dates ($F = 7.35$; df = 5, 36; $P < 0.01$), the temporal pattern of bug capture was differ-

Table 2. Plant parameters and numbers of marked and unmarked adult *L. hesperus* collected per 10-sweep samples from a late-April planting of Pima cotton

Date	Plant ht (mean ± SE)	No. nodes (mean ± SE)	Canopy width (mean ± SE)	Growth stage (median)	Marked bugs (mean ± SE)	Unmarked bugs (mean ± SE)
10 June	30.6 ± 0.63	9.8 ± 0.13	30.3 ± 0.53	Pinhead square	6.0 ± 0.66a	2.0 ± 0.49b
17 June	30.8 ± 0.68	10.7 ± 0.16	34.3 ± 0.66	Pinhead square	5.2 ± 0.66a	1.7 ± 0.49b
25 June	37.4 ± 0.83	11.6 ± 0.22	38.0 ± 0.70	Matchhead square	4.9 ± 0.66ab	1.0 ± 0.49b
1 July	42.4 ± 1.14	13.4 ± 0.28	44.5 ± 0.95	1/3-grown square	3.6 ± 0.66ab	2.0 ± 0.49b
8 July	49.3 ± 1.26	14.2 ± 0.27	40.6 ± 1.18	1/3-grown square	2.3 ± 0.66b	1.2 ± 0.49b
22 July	61.5 ± 1.32	18.2 ± 0.32	53.8 ± 1.47	Boll	3.7 ± 0.66ab	4.7 ± 0.49a

Means in a column followed by the same letter are not significantly different ($P > 0.05$; SIMULATE option of the LSMEANS statement in SAS).

Table 3. Plant parameters and numbers of marked and unmarked adult *L. hesperus* collected per 10-sweep samples from an early-June planting of Acala cotton

Date	Plant ht (mean ± SE)	No. nodes (mean ± SE)	Canopy width (mean ± SE)	Growth stage (median)	Marked bugs (mean ± SE)	Unmarked bugs (mean ± SE)
29 July	36.9 ± 0.68	10.4 ± 0.12	26.0 ± 0.73	Matchhead square	4.7 ± 0.61	0.3 ± 0.27b
5 Aug.	49.2 ± 0.88	12.3 ± 0.16	38.3 ± 0.85	1/3-grown square	4.0 ± 0.61	1.0 ± 0.27ab
12 Aug.	57.3 ± 1.08	13.8 ± 0.18	37.6 ± 0.79	1/3-grown square	2.7 ± 0.61	1.6 ± 0.27a
19 Aug.	59.2 ± 1.12	14.0 ± 0.18	40.7 ± 1.09	Boll	3.0 ± 0.61	0.4 ± 0.27b

Means in a column followed by the same letter are not significantly different ($P > 0.05$; SIMULATE option of the LSMEANS statement in SAS).

ent from that observed for marked bugs (Table 2). The number of captured native lygus was significantly higher on the last sample date (22 July) compared with earlier dates. As for the marked bugs, the sampler by date interaction ($F = 0.76$; df = 10, 36; $P = 0.66$) indicated the temporal pattern of collections of native lygus was similar among samplers.

Experiment 2, Acala Cotton. Conditions at the time of the second planting of Acala cotton facilitated vigorous vegetative growth. The first pinhead squares were generally observed at mainstem nodes 8 and 9, and early square retention was relatively low. Average plant heights during the study period ranged from ≈37–60 cm. Canopy widths were considerably less than plant heights (Table 3; height:width ranged from 1.30 to 1.53), resulting in an erect and narrow plant structure with few lower fruiting branches. As in the first experiment, fruit tended to be concentrated in the upper portions of the plant canopy on most sample dates.

No differences were observed among samplers in the mean numbers of marked lygus collected per 10-sweep sample ($F = 0.69$; df = 2, 24; $P = 0.51$). Absence of a significant sampler by date interaction ($F = 1.12$; df = 6, 24; $P = 0.38$) indicated the lack of differences among samplers was consistent across sample dates. In contrast with the first experiment, no temporal pattern in the collection of marked lygus was detected ($F = 2.26$; df = 3, 24; $P = 0.11$; Table 3). However, analyses of the numbers of marked bugs recovered from 1-m rows indicated differences among sample dates ($F = 6.64$; df = 3, 4; $P < 0.05$). Mean numbers of marked bugs recovered alive from 1-m row sections ranged from 6.5 (54%, 29 July and 19 August) to 11.0 (92%, 5 August). These differences were not statistically significant once adjusted for multiplicity (minimum observed adjusted $P = 0.06$). Estimates of sweep net collection efficiency were somewhat less variable compared with the first experiment in Acala cotton (range, 30.7 ± 2.69%, 29 July to 17.5 ± 2.69%, 12 August). It is unclear whether the slightly less variability observed in the second experiment should be attributed to loss of some marked bugs from the sample rows or to the narrower range of plant heights sampled.

Analyses of collections of naturally occurring lygus also indicated no significant influence of sampler ($F = 1.39$; df = 2, 22; $P = 0.27$) and no significant sampler by date interaction ($F = 0.29$; df = 6, 22; $P = 0.93$). However, a temporal pattern in the collection of native unmarked lygus was detected ($F = 5.30$; df = 3, 22; $P < 0.01$; Table 3). The numbers of native lygus collected per 10-sweep sample peaked on 12 August and were lowest on the first and last sample dates.

Experiment 2, Pima Cotton. Fruiting in the second planting of Pima also was delayed, and the median phenological stage of development did not advance beyond 1/3-grown square during the study (Table 4). Although plant heights were comparable with those in the Acala cotton, canopy widths were generally greater and the canopy structure was less erect (height:width ranged from 1.05 to 1.25).

No differences among samplers were observed in the numbers of marked lygus collected by the sweep net ($F = 0.88$; df = 2, 22; $P = 0.43$), and this lack of differences was consistent across sample dates (sampler by date interaction, $F = 0.80$; df = 6, 22; $P = 0.58$). Differences in the numbers of marked lygus collected were observed among sample dates ($F = 3.80$; df = 3, 22; $P = 0.02$). More marked bugs were collected during the 5 August sampling than on 12 August (Table 4), whereas counts on other dates were intermediate to these extremes. In contrast, mean numbers of bugs recovered alive from 1-m row sections did not differ among sample dates ($F = 1.13$; df = 3, 4; $P = 0.44$), suggesting the retention of marked bugs in sample

Table 4. Plant parameters and numbers of marked and unmarked adult *L. hesperus* collected per 10-sweep samples from an early-June planting of Pima cotton

Date	Plant ht (mean ± SE)	No. nodes (mean ± SE)	Canopy width (mean ± SE)	Growth stage (median)	Marked bugs (mean ± SE)	Unmarked bugs (mean ± SE)
29 July	36.6 ± 0.59	11.1 ± 0.16	35.1 ± 0.60	Matchhead square	3.6 ± 0.60ab	1.0 ± 0.25
5 Aug.	45.0 ± 0.90	12.2 ± 0.20	39.5 ± 0.81	1/3-grown square	4.6 ± 0.60a	0.4 ± 0.25
12 Aug.	58.2 ± 1.03	15.2 ± 0.17	48.3 ± 0.98	1/3-grown square	1.9 ± 0.60b	0.4 ± 0.25
19 Aug.	56.2 ± 1.12	14.8 ± 0.22	45.4 ± 0.94	1/3-grown square	3.2 ± 0.60ab	0.7 ± 0.25

Means in a column followed by the same letter are not significantly different ($P > 0.05$; SIMULATE option of the LSMEANS statement in SAS).

Table 5. Comparison of plant parameters as proxies for a main effect of date in analyses of the influence of sampler on collections of marked and released *L. hesperus* adults in Acala and Pima cotton

Statistic	Sampler	Date	Sampler × date	Sampler	Plant ht	Sampler × ht	Sampler	Plant width	Sampler × width	Sampler	H/W[a] ratio	Sampler × H/W
Acala cotton test 1												
F	0.44	3.86	1.27	0.01	3.85	0.03	0.13	2.46	0.05	0.51	0.06	0.62
df	2, 32.3	5, 24.7	10, 32.3	2, 44.6	1, 47.3	2, 44.7	2, 44.5	1, 47.4	2, 44.6	2, 46.9	1, 46.2	2, 46.9
P	0.65	0.01	0.29	0.99	0.06	0.97	0.88	0.12	0.95	0.60	0.81	0.54
Acala cotton test 2												
F	0.69	2.26	1.12	3.38	6.84	4.05	1.82	4.30	2.32	0.81	0.02	0.86
df	2, 24	3, 24	6, 24	2, 30	1, 30	2, 30	2, 30	1, 30	2, 30	2, 30	1, 30	2, 30
P	0.51	0.11	0.38	0.05	0.01	0.03	0.18	0.05	0.12	0.45	0.88	0.43
Pima cotton test 1												
F	0.75	4.14	1.02	1.91	9.89	1.85	2.71	5.82	2.81	0.86	7.58	0.75
df	2, 36	5, 36	10, 36	2, 48	1, 48	2, 48	2, 48	1, 48	2, 48	2, 48	1, 48	2, 48
P	0.48	<0.01	0.44	0.16	<0.01	0.17	0.08	0.02	0.07	0.43	<0.01	0.48
Pima cotton test 2												
F	0.88	3.80	0.80	1.78	6.65	1.57	1.38	3.89	1.38	1.37	2.10	1.24
df	2, 22	3, 22	6, 22	2, 30	1, 30	2, 30	2, 30	1, 30	2, 30	2, 30	1, 30	2, 30
P	0.43	0.02	0.58	0.19	0.02	0.22	0.27	0.06	0.27	0.27	0.16	0.30

In each analysis the response variable was number of marked *L. hesperus* adults captured in 10 sweeps. Fixed effects were sampler, date (or the plant parameter serving as a proxy for date), and their interaction. Block is a random effect.

[a] H/W ratio is the ratio of plant height to plant width.

rows was similar among dates. Mean numbers of marked bugs recovered alive from 1-m row sections ranged from 7.5 (62%, 19 August) to 9.5 (79%, 29 July and 5 August). As in the Acala cotton, calculated collection efficiency of the sweep net in the second experiment in Pima cotton (range, 30.3 ± 2.71, 5 August to 12.4 ± 2.99%, 12 August) tended to be somewhat lower and less variable than in the first experiment.

Collections of naturally occurring unmarked lygus also failed to indicate significant effects of sampler ($F = 0.20$; df = 2, 24; $P = 0.82$) or a sampler by date interaction ($F = 0.87$; df = 6, 24; $P = 0.53$). In contrast to marked bugs, no differences in the mean counts of unmarked native bugs were observed among sample dates ($F = 1.12$; df = 3, 24; $P = 0.36$; Table 4).

Analyses Using Plant Measurements as a Proxy for Date. Analyses indicated the ratio of plant height to canopy width significantly influenced collections of marked lygus in only one of the four experiments (experiment 1, Pima; Table 5). Lack of a significant effect by this plant parameter is probably explained by the narrow range in values among dates, and the absence of a consistent temporal trend in this ratio.

Unlike the plant height:width ratio, both plant height and canopy width varied more predictably with increasing calendar date. Significant effects of canopy width were indicated in both the first experiment in Pima cotton and in the second experiment in Acala cotton (Table 5). In contrast, significant effects of sample date were observed in all but the second experiment in Acala cotton (Table 5). Analyses indicated plant height influenced collections of marked bugs in three of the four experiments (Table 5). However, indicated effects of plant height were consistent with the effects of sample date in only two of the four experiments. In addition, the analysis using plant height for the second experiment in Acala cotton suggested significant effects for both sampler and the

sampler by plant height interaction (Table 5). In summary, none of the analyses using a plant parameter as a proxy for sample date returned results that were completely consistent with those of analyses using date as a fixed effect.

Discussion

Sampling studies using mark–release–recapture methods for calibration or estimation of collection efficiency implicitly assume that marked insects are captured at rates similar to those of naturally occurring insects. This assumption can be broken down into two separate issues: 1) whether released insects display the same behaviors as members of the wild population and 2) whether any differences in behavior result in altered capture rates. Regarding the first issue, Spurgeon (2009) observed that released bugs tended to move from the plants to which they had been introduced. Cooper and Spurgeon (2010a) evaluated the influence of time of release, relative to the time of sampling, on capture rates of marked adult lygus. They concluded that differences in captures of marked adult lygus corresponding to times of release resulted at least in part from redistribution of marked bugs within the cotton canopy. In conjunction with this latter report, field observations suggested that released bugs displayed a diel periodicity in within-plant distribution that was similar to that of the native population (our unpublished observation). This suggestion was based on observations of adults (both released and naturally occurring) in the upper plant canopy during the early morning hours, and their apparent absence later in the day. Finally, preliminary sampling studies have indicated that marked and released prereproductive female adult lygus are captured by the sweep net less frequently than are prereproductive males or reproductive adults of either gender (our unpublished data). Whereas these ob-

servations do not guarantee the behaviors of marked and released bugs are the same as those of the native population, they suggest similarities in behavior.

Regarding the second issue (whether altered behavior influences capture by the sweep net), Wilson et al. (1984), Snodgrass (1998), and Rosenheim et al. (2004) reported different patterns of within-plant distribution or plant part association for lygus nymphs and adults. However, Zink and Rosenheim (2004) reported that collection efficiency of the sweep net for late-instar lygus nymphs was nearly equivalent to that for adults. Therefore, the differences in behaviors between late-instar nymphs and adults were apparently insufficient to cause large differences in collection efficiency. Considering that we have observed marked adults to feed, mate, and oviposit (our unpublished observations), it seems likely that differences between marked and native adults in susceptibility to capture by the sweep net would be smaller than differences between native adults and nymphs. Therefore, whether marked and released adult lygus display the exact repertoire of behaviors displayed by native adults may be of little consequence. Regardless, results reported herein are not as subject to assumptions about the behavior of marked and released adults as are results of studies to estimate collection efficiency. Instead, our results only require that any changes in collection efficiency resulting from altered behavior of marked adults be reflected similarly in the counts collected by different samplers.

The lygus population levels we used were substantially higher than those corresponding to treatment thresholds commonly used in the West. Godfrey et al. (2010) recommend insecticide treatment of lygus in California cotton at population levels of two to four adults per 50 sweeps (0.4–0.8 per 10 sweeps) during the early squaring period, and 7–10 adults per 50 sweeps (1.4–2 per 10 sweeps) during the period from first bloom to first boll. The recommended treatment threshold in Arizona is 15 adult lygus plus four nymphs per 100 sweeps (Ellsworth 2001). Based on our captures, collections of both marked and naturally occurring lygus were well above either California or Arizona thresholds on most sample dates. However, use of lygus population levels closer to established thresholds in our study would have minimized the range of possible captures among samplers or dates and would probably have obscured any differences that were present. In addition, Spurgeon (2009) showed the relationships between sweep net captures of marked adult lygus and actual population densities, once adjusted for plant size class, were essentially linear over a wide range of population densities (one to six marked adults per row m). Therefore, results from our study should be relevant to the lower population levels represented by commonly used treatment thresholds.

We evaluated the influence of sampler on counts of *Lygus hesperus* adults collected using the standard sweep net in both Acala and Pima cottons representing a variety of plant sizes and stages of development. At no time did we observe any statistical evidence of a sampler effect based on marked and released bugs or

on the native lygus population. This finding clearly indicates if the sweeping technique and sampling pace is carefully coordinated among different samplers, the variation in population estimates that is attributable to sampler is relatively small compared with other sources of variation. Implicit in this finding is that the samplers are physically capable, motivated, and adequately trained to perform the sweeping technique. Given that these conditions are satisfied, our results demonstrate that different samplers can use the sweep net to obtain equivalent estimates of adult lygus populations in cotton.

We also observed temporal patterns in lygus collections corresponding to sample date. In the first experiment, counts of marked lygus adults tended to be highest on the earliest sample dates, which corresponded to the smallest and least developed plants. This observation is consistent with the report of Spurgeon (2009), who documented a plant-size effect on the collection efficiency of the sweep net. However, temporal patterns in lygus collections during the second experiment deviated somewhat from this pattern. During the second experiment, we regularly observed predation by assassin bugs (Hemiptera: Reduviidae) on both marked and unmarked lygus (our unpublished data). Therefore, predation in the second experiment seems the most likely explanation for the observed differences in temporal population patterns between experiments 1 and 2.

Spurgeon (2009) reported the sweep net collected ≈21% of marked adult lygus from plants <50 cm in height, but collection efficiency declined in association with taller plants. In the current study, we observed much higher collection efficiencies on the earliest sample dates, and less severe declines in sampling efficiency when plants exceeded 50 cm in height. Analyses of selected plant parameters did not yield covariates with effects equivalent to, or consistent with, the effect of sampling date. Although plant height is clearly associated with changes in collection efficiency by the sweep net, it does not seem adequate by itself to explain variations in collections among sample dates. Wilson et al. (1984) suggested that production of lateral branches facilitated the movement of lygus away from the upper nodes of the plants. In that case, we would have expected obvious differences in collections of marked lygus between Acala and Pima cotton varieties on the same dates, by virtue of the more extensive branching exhibited by the Pima variety we used. Because we did not observe these differences we hypothesize the major differences in collection efficiencies observed in our study compared with those observed by Spurgeon (2009) may be related to differences in fruiting patterns between the two studies. Additional study to examine this hypothesis is warranted.

Relatively high population levels of unmarked (naturally occurring) lygus bugs were present during most samplings of both studies, and differences in the numbers of these native lygus were observed among sample dates in two of the tests. Whether or not differences in counts of native bugs were observed among

dates, the observed temporal patterns were generally inconsistent with the patterns observed for marked bugs. High levels of predation in experiment 2 may have influenced the observed temporal patterns of counts of marked bugs, rendering comparisons with such patterns of native bugs irrelevant. However, retention of marked adults in experiment 1 was generally high across sample dates. In experiment 1, counts of marked lygus collected with the sweep net tended to decrease with increasing plant development in the first weeks of the study, whereas counts of native lygus either did not change (Acala) or exhibited a pattern different from that of marked bugs (Pima). These observations suggest that changes in sweep net sampling efficiency corresponding to increasing plant development may have obscured the true temporal patterns of the native lygus population. Thus, the size of the naturally occurring lygus population was increasingly underestimated with increasing plant development. This finding may be of critical importance to ecological studies and to efforts to develop improved treatment thresholds, and warrants continued study to elucidate the plant-based factors influencing collection efficiency of lygus adults by the sweep net.

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